# Exogenous factors influencing voice prosthetic biofilm

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# Chapter 8

# Caffeinated soft drinks reduce bacterial prevalence in voice prosthetic biofilms

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## Introduction

Silicone rubber tracheo-oesophageal voice prostheses, placed in a surgically created fistula in between the trachea and the oesophagus, are used by laryngectomized patients to regain their voice and speech. By closing the tracheostoma with a finger, air is conducted through the valve of the prosthesis into the neopharynx, where remaining mucosal and muscular structures are brought into vibration. After 3–4 months,[1,2] the voice prostheses usually fail because of the formation of a biofilm on the oesophageal side of the prosthesis, consisting of a multitude of bacterial and yeast strains.[3,4,5] Yeasts, notably *Candida albicans* are mainly held responsible for the dysfunction of the voice prosthesis, but also streptococci are believed to cause leakage, while enterococci are held responsible for increased airflow resistance.[6] Recenly *Rothia dentrocariosa* (Elving *et al.*, personal observations) have been suggested as causative organisms for prosthesis failure. Tracheo-oesophageal shunt speech, together with oesophageal injection speech and the use of an electrolarynx, is the most succesful method of speech restoration, leading to a high quality of speech within 14 days post-operatively.[7] Augmentation of the lifetime of voice prostheses would improve the quality of life of laryngectomized patients, as well as lead to savings on health care.

Current practice in otorhinolaryngology to prolong the lifetime of voice prostheses includes controversial, long-term prescription of antimycotics, although a clinical effect has not been proven.[2,8] In an era in which antibiotic and antimycotic resistance among microorganisms is rapidly emerging, the otorhinolaryngologist thereby contributes to the development of microbial resistance.[9] Alternatively, among laryngectomized patients, anecdotal

evidence circulates about the potential beneficial effects of buttermilk, Turkish yoghurt and caffeinated soft drinks. Scientific evidence is difficult to obtain in clinical studies, because biofilm formation on voice prostheses is governed by a multitude of environmental and patients life style-related conditions that are difficult to control over extended periods of time. In order to evaluate the effects of isolated, single factors on the development of biofilms on voice prostheses, an artificial throat[10] has been designed, i.e. a modified Robbins device, in which a number of voice prostheses could be fitted. Essential to establish clinically relevant biofilms on voice prostheses in the artificial throat, is a so-called teleological biofilm model.[11] In this approach, the entire microflora from an explanted voice prosthesis is used to inoculate the device, without isolation and identification of the micro-organisms composing the flora. A second important feature in the design of a relevant biofilm is that the colonizing organisms are passed through cycles of feast and famine[12], which is essential to stimulate the colonizing yeasts to grow into the silicone rubber prosthesis material.

The aim of this study is to investigate the influence of caffeinated soft drinks, as an isolated single factor, on the formation and microbial composition of a biofilm on silicone rubber voice prostheses in an artificial throat.

## Materials and methods

*Voice prostheses*

"Low Resistance" Groningen voice prostheses were supplied by Medin Instruments and Supplies (Groningen, The Netherlands) and Provox II voice prostheses were provided by Atos Medical AB (Hörby, Sweden). The low-resistance Groningen voice prosthesis (Figure 1A) consists of a tube with two flanges and a semicircular slit of 145° in the hat of the oesophageal flange, functioning as an one-way valve. The Provox II voice prosthesis (Figure 1B) is a biflanged device. The valve is moulded into one piece with the prosthesis and supported by a radiopaque, fluoroplastic ring, which is fastened in the shaft of the prosthesis. Both types are made of medical grade silicone rubber and only differ in their design.

*Soft drinks*

The caffeinated soft drinks used are listed in Table 1, together with their pH and sugar content. A decaffeinated and a sugar-free version of one caffeinated soft drink were included. As controls phosphate buffered saline (PBS) and carbonated mineral water (CMW) were used.

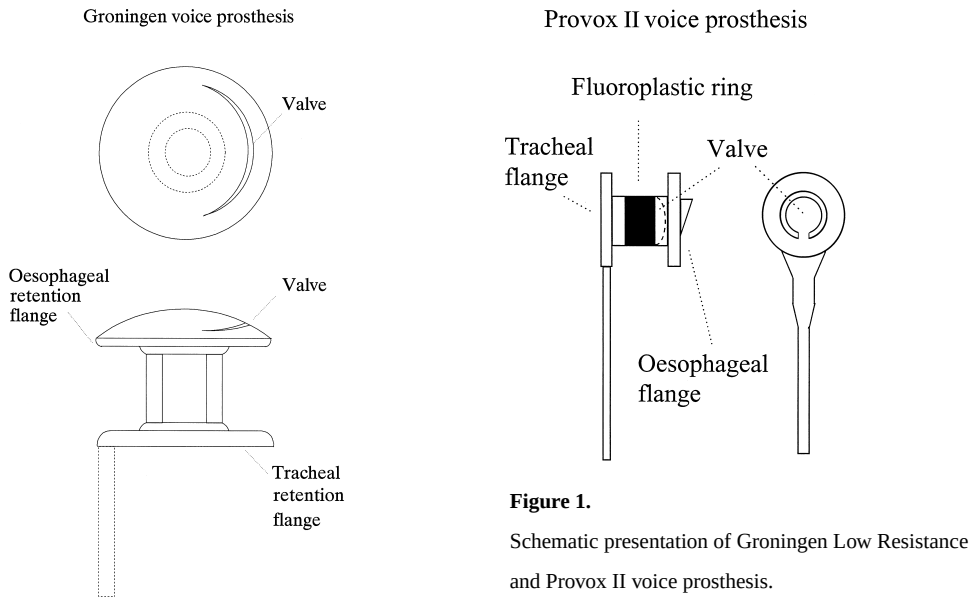


**Figure 1.**

Schematic presentation of Groningen Low Resistance and Provox II voice prosthesis.

*Biofilm formation in the artificial throat*

The voice prostheses were placed in two transparant modified Robbins devices, described as artificial throats.[10] Each was equipped with three Groningen and two Provox II voice prostheses. The third Groningen voice prosthesis was used for electron microscopy. The total cultivable microflora from an explanted Groningen voice prosthesis, containing a variety of yeasts and bacterial strains, among which *Candida albicans*, *Candida tropicalis*, streptococcal and staphylococcal strains, was cultured in a mixture of 30% brain heart infusion broth (OXOID, Basingstoke, Great Britain) and 70% defined yeast medium (per liter: 7.5 g glucose, 3.5 g $(NH_4)_2SO_4$, 1.5 g L-asparagine, 10 mg L-histidine, 20 mg DL-methionine, 20 mg DL-tryptophane, 1 g $KH_2PO_4$, 500 mg $MgSO_4.7H_2O$, 500 mg NaCl, 500 mg $CaCl_2.2H_2O$, 100 mg yeast extract, 500 g $H_3BO_3$, 400 g $ZnSO_4.7H_20$, 120 g $FE(III)Cl_3$, 200 g $Na_2MoO_4.2H_2O$, 100 g KI, 40 g $CuSO_4.5H_2O$) and used to inoculate the artificial throats during 5 hours. Subsequently, a biofilm was allowed to grow on the voice prostheses over 3 days by filling the devices with growth medium. On the fourth day, the devices were perfused with 650 ml phosphate buffered saline (10mM potassium phosphate and 150 mM NaCl, pH 7.0), serving as a control, or a soft drink, after which the prostheses were left in the moist environment of the drained artificial throats. This perfusion scheme was repeated three times a day over nine days. At the end of each day all artificial throats were filled with growth medium for half an hour and left overnight after draining. The experiments lasted fifteen days in

**Table 1.** Soft drinks used, with their pH and sugar content.

| Soft drink | PH | Sugar content | Source |
|---|---|---|---|
| PBS, control | 7.0 | No sugar | |
| Carbonated mineral water | 3.7 | No sugar | Spa Rood, Spa Monopole N. V., Spa, Belgium. |
| Caffeinated soft drink I | 2.6 | Sugar | Coca-Cola, The Coca-Cola company, bottled by Coca Cola Enterprises Nederland B.V., Schiedam, The Netherlands. |
| Caffeinated soft drink II | 2.5 | Sugar | Pepsi Cola, Pepsico Inc. New York, U.S.A., bottled by Vrumona B.V. Antwerpen, Belgium. |
| Decaffeinated soft drink | 3.2 | No sugar. Replaced by aspartaam. and acesulfaam – K | Decaffeinated Coke Light, The Coca-Cola company, bottled by Coca Cola Enterprises Nederland B.V., Schiedam, The Netherlands. |
| Sugar free caffeinated softdrink | 3.2 | No sugar. Replaced by aspartaam and acesulfaam – K. | Coca-Cola Light, The Coca-Cola company, bottled by Coca-Cola Enterprises Nederland B.V. Schiedam, The Netherlands |

total and were performed at room temperature. The tracheal sides of the prostheses were left in ambient air without taking precautions to prevent infections from the air, similar to the situation with a stoma.

*Evaluation of biofilms*

On the fifteenth day of the experiment the voice prostheses were removed from the artificial throats after a final perfusion with 200 ml PBS. Subsequently, the biofilm was removed from the valve sides of the prostheses by scraping and, after sonication (60 s), serially diluted in reduced transport fluid (RTF) (per liter: 75 ml of Solution I (1.2% NaCl, 1.2% $(NH_4)_2SO_4$, 0.6% $KH_2PO_4$, 0.51% $MgSO_4.7H_2O$); 75 ml of Solution II (0.6% $K_2HPO_4$); 10 ml of 3.72% EDTA; 20 ml of 1% cysteine HCl and 820 ml of distilled water). The microbial compositions of the biofilms were evaluated by plating the serial dilution on MRS (de Man, Rogosa and

Sharpe) agar plates for yeasts, and blood agar plates for bacteria. After two days at 37° C in an aerobic incubator, the numbers of bacterial and fungal colony forming units per ml (CFU/ml) were determined for each prosthesis separately and expressed as a percentage number with respect to the control. The consistency of the results was secured by comparison with the control throat.

*Scanning electron microscopic evaluation*

Electron microscopy was only done on Groningen voice prostheses as, due to their design, biofilm features can be more easily assessed and compared on Groningen than on Provox II voice prostheses. Biofilm covered voice prostheses were flushed with 6.8% sucrose and 0.1 M cacodylate buffer (pH 7.4), fixed and stained in 2% glutardialdehyde and 0.2% ruthenium red in 0.1 M cacodylate buffer at 4° C and flushed again. Post-fixation and staining was carried out in 1% $OsO_4$ and 0.2% ruthenium red in cacodylate buffer by gently shaking for 3 h at room temperature. Buffer washes and dehydration involved the following rinsing procedures: 20 min in 6.8% sucrose in 0.1 M cacodylate buffer; 3 x 10 min bidistilled water; 20 min in respectively 30, 50 and 70% ethanol and 4 x 30 min in 100% ethanol. After critical-point drying with $CO_2$ for 4 h, the specimens were mounted on SEM stubs and sputter-coated with gold/paladium (15 nm). SEM observations were taken, made using the JEOL 6301, with different magnifications at 15-25 kV.

*Microbial growth inhibition test*

To study the direct effect of the soft drinks used on oropharyngeal yeasts and bacteria a growth inhibition test was performed as described by Elving et al.[13] *Candida albicans, Candida humicola* and *Rothia dentrocariosa*, *Staphylococus epidermidis* and *Streptococcus vestibularis*, all voice prosthetic isolates, were cultured overnight and harvested by centrifugation and diluted in RTF to a concentration allowing confluent growth when plated with a cotton swab on the agar. Yeasts were plated on MRS, while bacteria were plated on brain heart infusion agar. Agar plates were dried for 20 min at room temperature and 5 μl of soft drink were spotted onto the surface of the agar plate. After overnight incubation, the agar plates were screened for growth inhibition zones around the soft drink spots.

## Results

After perfusing an artificial throat intermittently over 9 days three times a day with 650 ml of caffeinated soft drink I (see Figure 2), the number of bacteria in the biofilm on Groningen and

Provox II voice prostheses was reduced to 1% of the number of bacteria found when perfusing an artificial throat with PBS. The amount of yeasts in the biofilm was 116% and 81% of the control, respectively. Caffeinated soft drink II reduced the amount of bacteria on the Groningen prostheses to 5% and on the Provox II voice prosthesis to 4% of the control, while the amount of yeasts were 172% and 197%, respectively.

Perfusion of an artificial throat with a decaffeinated or sugar-free version of one of the soft drinks did not have this inhibitory effect on bacterial prevalence in the biofilm on either type of prostheses (see also Figure 2). In addition, CMW, used as a second control, did not show any inhibitory effect on the Groningen prostheses, while it reduced bacteria on Provox prostheses to 41% of the control.

Figure 3 shows the surface of a Groningen voice prosthesis taken from the artificial throat perfused with caffeinated soft drink I. Patches of biofilm are visible, while the high magnification shows predominantly yeasts.

In an agar diffusion test, it was separately assessed, that none of the soft drinks showed any growth inhibition of oropharyngeal yeasts (*Candida albicans* and *Candida humicola*) or bacteria (*Rothia dentrocariosa, Staphylococcus epidermidis* and *Streptococcus vestibularis*).

## Discussion

Consumption of excessive amounts of soft drinks, notably caffeinated soft drinks has been associated with both health and disease. Caffeinated soft drinks are well known among travellers to have a positive effect on diarrhoea as an alternative for oral rehydration salts and can be used to enhance resorption of medication. On the other hand, erosion of teeth may occur after daily consumption of excessive amounts of caffeinated soft drinks.[14] In addition, effects on feeding tube clogging and on bolus obstruction in oesophageal strictures are reported, as well as a spermacidal effect, when using caffeinated soft drinks as a post coitum rinse.[15,16,17]

The present study investigates the influence of caffeinated soft drinks, as an isolated single factor, on the formation of a biofilm on voice prostheses *in vitro*, as well as the prevalence of bacteria and yeasts in these biofilms. Previously, dietary factors such as the consumption of buttermilk[18] and other dairy products with probiotic bacteria[19,20] have been demonstrated to have an inhibitory effect on the formation of voice prosthetic biofilms in an artificial throat. In the present study only caffeinated soft drinks reduced bacterial prevalence in the biofilms, while yeasts thrived. The small difference in effects between the two types of voice prostheses is most likely due to differences in design. Neither the decaffeinated soft drink (pH 3.2)

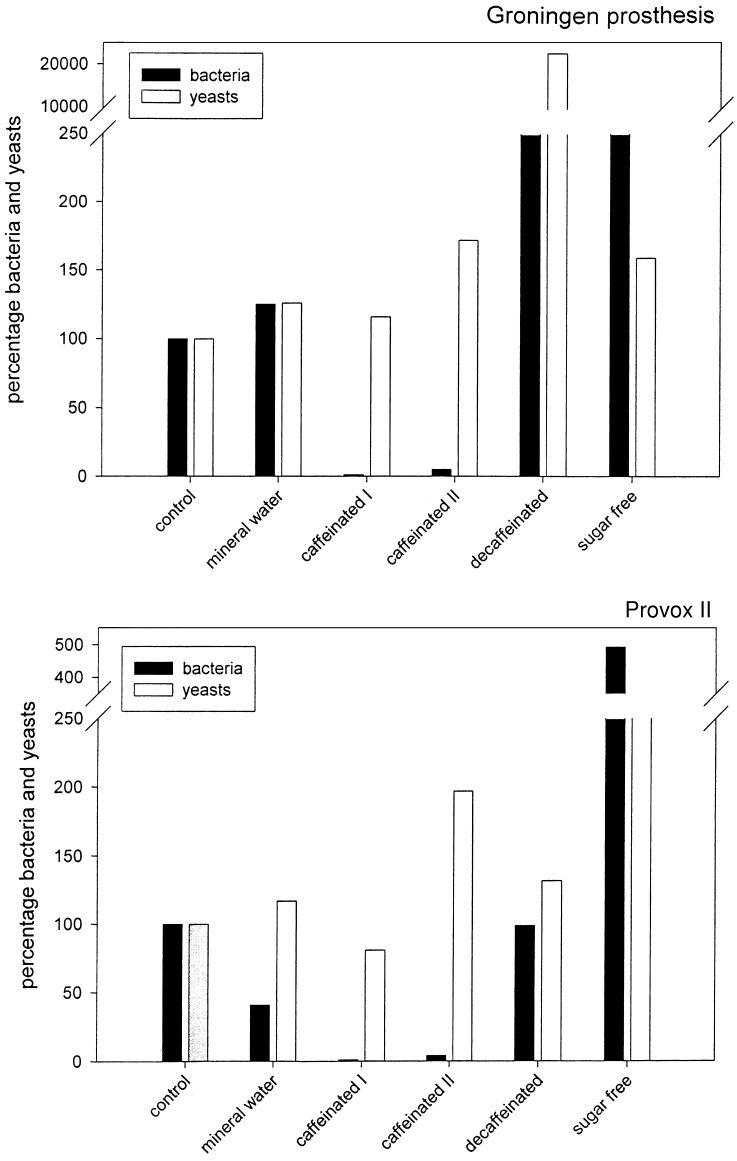


**Figure 2.**

The percentage of bacteria and yeasts isolated from silicone rubber voice prostheses in an artificial throat, after daily perfusion with caffeinated softdrinks. Results are expressed relative to phosphate buffered saline, as a control set at 100%. The experiments with caffeinated soft drink I was performed four times, all other experiments were conducted in duplicate, with two prostheses of each type included per run. Correspondence in the absolute values of microbial prevalence between runs was better than 5%.

with a slightly higher pH than caffeinated soft drink I (pH 2.6), nor the sugar-free version at pH 3.2 (see Table 1) reduced the number of bacteria in the biofilm. This suggests that caffeine, or a combination of a low pH and high sugar content, may be essential to the inhibitory effects measured, although no inhibitory effects were measured of the caffeinated soft drinks in an agar diffusion test. Alternatively, the caffeinated soft drinks may decrease the cohesivity of the biofilms causing detachment during the perfusion steps, although it is hard

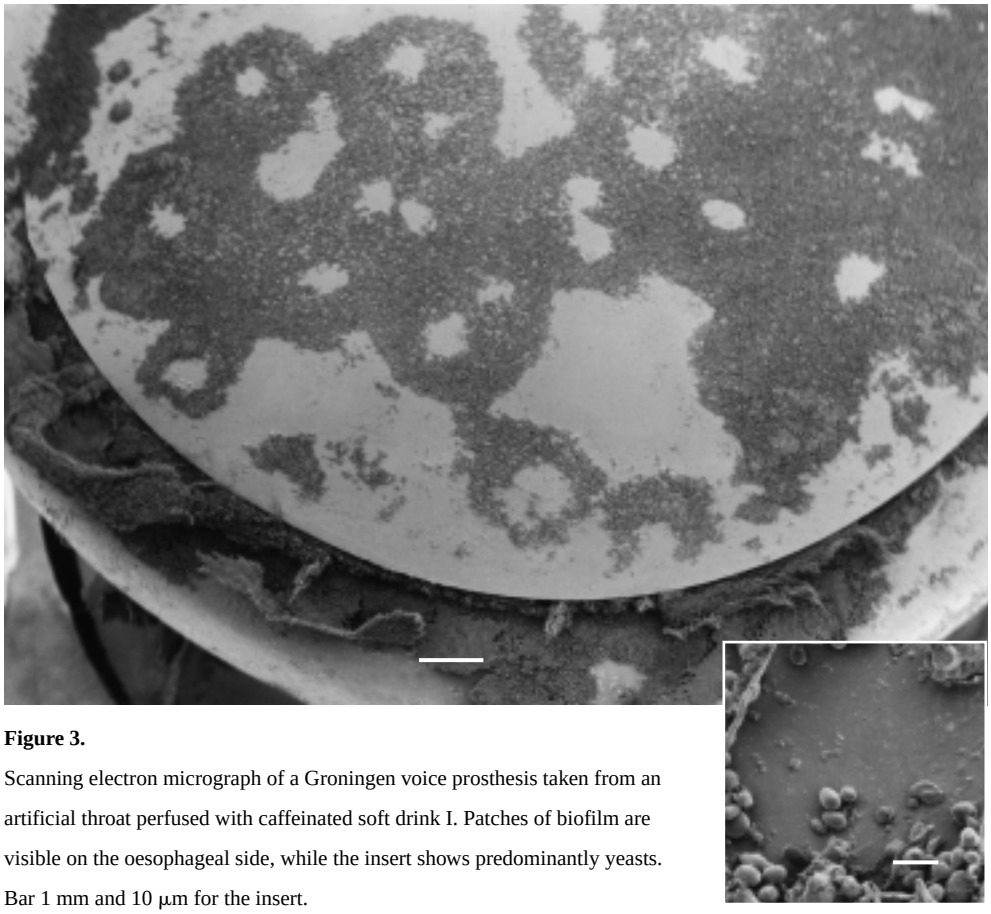

**Figure 3.**

Scanning electron micrograph of a Groningen voice prosthesis taken from an artificial throat perfused with caffeinated soft drink I. Patches of biofilm are visible on the oesophageal side, while the insert shows predominantly yeasts. Bar 1 mm and 10 μm for the insert.

to imagine why this would only affect bacterial prevalence.

Yeasts are believed to be mainly responsible for the disfunctioning of voice prostheses,[3,4,5,21] but Parket et al.[6] suggested that certain bacterial strains are involved as well, in which case the use of caffeinated soft drinks by patients could help augment the lifetime of their voice prostheses through their effects on bacterial prevalence.

In summary, this *in vitro* study shows that caffeinated soft drinks reduce bacterial prevalence in voice prosthetic biofilms. The relevance of the present results for laryngectomized patients has to be established in a clinical trial, which might be difficult because of the multiple factors influencing biofilm formation on voice prostheses *in vivo.* However, this study points to rather simple alternatives to antibiotics and antimycotics to influence biofilm formation on voice prostheses and prolong their lifetime through the incorporation of caffeinated soft drinks in a carefully designed diet for laryngectomees.

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